SUN YAT-SEN UNIVERSITY,

ZHONGSHAN SCHOOL OF MEDICINES

APPLICATION FORM FOR ETHICAL REVIEW OF WELFARE AND ETHICS OF EXPERIMENTAL ANIMALS

<table>
<tr><td colspan="4">Protocol name Tracing and modifying the homing capability of bone marrow derived mesenchymal stem cells</td></tr>
<tr><td colspan="4">Protocol source General Program, The National Natural Science Foundation of China</td></tr>
<tr><td colspan="2">Principle investigator
Xiang Peng</td><td colspan="2">Name of unit Key Laboratory for Stem Cells and Tissue Engineering</td></tr>
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<tr><td colspan="4">Experimental animal using permission No. SYXK（粤）2015-0107</td></tr>
<tr><td colspan="4">Experiment schedule: From July 1st, 2016 to October 1st, 2016</td></tr>
<tr><td colspan="4">Please provide the name and the permission number of previous censored projects and elaborate which are similar or identical to this project.
None</td></tr>
<tr><td colspan="4">Is the project correlate to previous censored projects? If so, write down the project name and the approval number.
No</td></tr>
<tr><td rowspan="2">Experimental animal</td><td colspan="2">Source:
Sun Yat-sen experimental animal center</td><td>Breed strain: BALB/c mice</td></tr>
<tr><td colspan="2">Level: SPF (√)
Normal ( )</td><td>Number of BALB/c mice: 28
(♂ 28)</td></tr>
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The purpose, method, significance and feasibility of the experiments.	Purpose: Separate, identify and engineer bone marrow derived mesenchymal stem cells (MSCs) in order to empower the homing ability of MSCs to local inflammatory sites of delayed type hypersensitivity and mark MSCs with fluorescent protein for tracing. Method: 1.EF-1α-CXCR5-IRES-eGFP plasmid and EF-1α -eGFP plasmid will be constructed and transfected respectively by lentivirus to human MSCs. 2.$MSCs^{EF\text{-}1\alpha\text{-}CXCR5\text{-}IRES\text{-}eGFP}$ that simultaneously express CXCR5 and eGFP and $MSCs^{EF\text{-}1\alpha\text{-}eGFP}$ expressing eGFP will be screened out by flow cytometry 3. 150μL 0.5% DNFB solution in acetone/olive oil (4:1) will be applied to the shaved backs of 20 mice. Seven days later, right ears will be challenged with 20μL 0.3% DNFB solution. An identical amount of acetone/olive oil (4:1) will be administered to the left ear as control. The control group of 4 mice will receive acetone/olive oil (4:1) on their backs and ears as control. 4. 2 hours after sensitization, a total amount of $1x10^6$ of $MSCs^{EF\text{-}1\alpha\text{-}CXCR5\text{-}IRES\text{-}eGFP}$, $MSCs^{EF\text{-}1\alpha\text{-}eGFP}$ and identical volume of PBS will be injected to the caudal veins of mice respectively. 5. Ear samples will be harvested 48 hours after sensitization. Samples will be analyzed after measurement, qPCR and immunofluorescence. All the animals will be sacrificed by cervical dislocation. The process of feeding, operation and management are in conformity with the detail rules for the implementation of laboratory biological safety management and experimental animal welfare ethics principle.

Significance:

Without involving ethic problems in clinical application, with a handful of side effect or concern of safety, MSCs are playing an important role in immuno-regulation and immune-reaction such as GVHD, autoimmune disease and so on. However, due to lack of chemokine receptors, MSCs are blamed for its low homing efficiency to inflammatory sites that in several cases MSCs failed to live up to its expected effect. Meanwhile, limited facts about the in vivo distribution of intravenous injected MSCs have been testified because of the difficulty to localize in living body. Thus, empowering MSCs with chemokine receptors and maker molecules to enhance their ability of homing and immune-regulation will not only contributes to improve the clinical efficacy as well as the prognosis of patients, but also sharpens the understanding of the distribution of intravenous injected MSCs in vivo.

Feasibility:

1. Full foundation

Our project is based on sufficient article reading and clinical information which prop up the evidence of distinct chemokines of specific organs, the immuno-regulatory function of MSCs, the positive outcomes of implement both in patients as well as in disease models of mouse and the in body marking function of Luciferase.

2. Lab

The lab condition and staff guarantee the actualization of the project. Our research is supported by Key Laboratory for Stem Cells and Tissue Engineering which is a center for Stem Cell Biology and Tissue Engineering, supported by Zhongshan School of Medicine, Sun Yat-sen University, Ministry of Education. With a Safety Level 2 laboratory of approximate 3500m^2 and equipment worth over 15 million, we are equipped with important and supportive platforms, such as stem cells bank and stem cell preclinical primate evaluation platform, as well as necessary equipment for the project such as BD-Influx flow cytometry、Illumina Solexa GAIIX gene sequencing instrument and laser scanning confocal microscope.

Feeding situation of experimental animals	Fed and managed by Sun Yat-sen experimental animal center
The design of the animal experiment	Experimental grouping: DTH Model (♂ 22) : DTH model with 22 BALB/c mice will be established and divided into 3 experiments: (1) Eight mice will be included in this experiment. 150μL 0.5% DNFB solution in acetone/olive oil (4:1) will be applied to the shaved backs of 20 mice. Seven days later, right ears will be challenged with 20μL 0.3% DNFB solution. After sensitization, $1x10^6$ $MSCs^{EF\text{-}1\alpha\text{-}CXCR5\text{-}IRES\text{-}eGFP}$ will be injected to the caudal veins. Ear samples of 4 mice will be harvested for qPCR and HE staining while that of the remaining 4 mice will be preserved for immune-fluorescence. (2) Eight mice will be included in this experiment. 150μL 0.5% DNFB solution in acetone/olive oil (4:1) will be applied to the shaved backs of 20 mice. Seven days later, right ears will be challenged with 20μL 0.3% DNFB solution. Following sensitization, $1x10^6$ $MSCs^{EF\text{-}1\alpha\text{-}eGFP}$ will be injected to the caudal veins. The ear samples will be treated as above. (3) Six mice will be included in this experiment. 150μL 0.5% DNFB solution in acetone/olive oil (4:1) will be applied to the shaved backs of 20 mice. Seven days later, right ears will be challenged with 20μL 0.3% DNFB solution. Following sensitization, identical volume of PBS will be injected to the caudal veins. The ear sample will be harvested for qPCR and HE staining. Control group (♂ 6) : (4) Six mice containing in this group will receive same volume of acetone/olive oil solution application to the backs and ears. Identical volume of PBS will be injected to the caudal veins. Ears samples will be treated as above.
	Administration mode: intragastric administration ( ) subcutaneous injection ( ) intraperitoneal injection ( ) intravenous injection (√) others ( ) Note if 'other' is chosen:

	Executing manner: cervical dislocation (√) overdose of anesthetic ( ) others ( ) Note if 'other' is chosen:
	The management of dead bodie: sanity treatment (√) others( ) Note if 'other' is chosen:
The embodiment of "3R" principle in designing the experiments	Replacement: In our design, we choose to culture bone marrow derived mesenchymal stem cell in vitro to substitute living animals.
	Reduction: In our design, we have reduced the number of animals used. We will establish DTH model of BALB/c mice which will be divided into 4 experiments. In the first three experiments will injected human $MSCs^{EF\text{-}1\alpha\text{-}CXCR5p\text{-}IRES\text{-}eGFP}$、human $MSCs^{EF\text{-}1\alpha\text{-}eGFP}$ and PBS to mice after sensitization while in the last group, mice will be injected with PBS after applying acetone/olive oil solution as control. In the following experiment, ear samples will be measured and harvested for immune-fluorescence, HE staining and qPCR which will contribute to the further analysis of the distribution of intravenous injected MSCs.
	Refinement: All the experiment technique required in our project, such as anesthesia, surgical operations and function test has been fully mastered. At the same time, the experimental technical route has been delicately designed and selected in order to reduce discomfort and suffer of experimental animals which will promise the repeatability of animal experiment.
Announcement: I will consciously abide by the experimental animal welfare ethics principle and agree to accept the supervision as well as inspection of commission and inspector of laboratory manager. If I violate the commitment or stipulation, I will voluntarily accept punishment. Signed (or stamped) by the announcer: Zhou Longyuan Date: 1st, August, 2016	
Review opinion of the declaring unit: Agree. Signed (or stamped) by the head executive investigator: A. P. Xiang Date 10th, August, 2016	

Instruction:

1. The principle and the head executive investigators of the project and the leader of cooperative departments are required to sign in the announcer's signature column.
2. materials for review shall be submitted with the application (a copy of electronic as well as written document). The issues involving animal welfare and ethics, such as significance, necessities, purpose and feeding management of experimental animal, the experiment operation, observation steps and methods need expatiation. Additional pages are acceptable.
3. Please print double-sided.